

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平7-184640

(43) 公開日 平成7年(1995)7月25日

(51) Int.Cl. <sup>5</sup>	識別記号	庁内整理番号	F I	技術表示箇所
C 1 2 N 1/16	Z	8828-4B		
C 0 2 F 3/28	Z			
3/34	Z			
C 1 2 N 9/14				
// (C 1 2 N 9/14				

審査請求 未請求 請求項の数 3 F D (全 4 頁) 最終頁に続く

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(54) 【発明の名称】 酵母エキス残渣の処理方法

(57) 【要約】

【目的】 酵母エキス残渣を処理する新規な方法を提供する。

【構成】 酵母溶解酵素生産菌の培養液を使用して、酵母エキス残渣を可溶化し、可溶化液を嫌気性排水処理法で処理することを特徴とする酵母エキス残渣の処理方法である。

【効果】 酵母溶解酵素生産菌の培養液を使用して安価で効率的な処理が可能である。

## 【特許請求の範囲】

【請求項 1】 酵母溶解酵素 (YLase) 生産菌の培養液を使用して酵母エキス残渣 (YCW) を可溶化し、可溶化液を嫌気性排水処理法で処理することを特徴とする酵母エキス残渣の処理方法。

【請求項 2】 請求項 1 記載の菌として酵母溶解酵素 (YLase) 高生産菌オエルスコフィア (Oerskovia sp. E24 (工業技術院生命工学工業研究所特許微生物寄託番号、FERM P-13692)) を使用することを特徴とする酵母エキス残渣処理方法。

【請求項 3】 請求項 1 記載の菌を培養する培地として、酵母エキス残渣を用いることを特徴とする YCW 処理方法。

## 【発明の詳細な説明】

## 【 0 0 0 1 】

【産業上の利用分野】 本発明は、オエルスコフィア (Oerskovia) に属する酵母溶解酵素 (YLase) 高生産菌 (Oerskovia sp. E24, FERM P-13692) を酵母エキス残渣 (YCW) を用いて培養し、その培養液により YCW を可溶化した後に、嫌気性排水処理法により処理することを特徴とする YCW の処理に関する。

## 【 0 0 0 2 】

【従来の技術および発明が解決しようとする課題】 従来技術による YCW の処理方法としては、(1) 海洋投棄又は埋め立てによる廃棄処分、(2) 焼却、(3) 直接嫌気性消化、(4) 酵母溶解酵素 (YLase) による可溶化および可溶化液の嫌気性排水処理が考えられるが、次のような問題点がある。(1) 海洋投棄は、将来禁止され、また、環境保護の観点から埋め立て処分も制限される方向にある。(2) 焼却は、排ガスの処理が必要であり、環境保護の観点から満足できるものではない。(3) 直接嫌気性消化は、下水汚泥等の有機固形物の処理に通常用いられるが、処理に時間がかかる (20~30 日)。(4) 酵母溶解酵素で可溶化することにより、高速メタン発酵処理装置 (UASB 等) で処理できることが従来技術から予想されるが、YCW の可溶化に市販酵素を用いると酵素の費用が高くなる場合がある。また、既知の酵母溶解酵素生産菌を用いることも考えられるが酵素生産用の培地が必要である。このように従来技術による YCW の処理には種々の問題があり、より安価で、効率的な処理方法の開発が必要である。

## 【 0 0 0 3 】

【課題を解決するための手段】 かかる問題を解決するために鋭意検討した結果、酵母溶解酵素高生産菌の培養液の使用により簡便に YCW を可溶化することができ、この可溶化液は嫌気性排水処理により処理できることを見いだした。用いた酵母溶解酵素高生産菌は、我々が酵母エキス製造用に開発した酵母溶解酵素高生産菌 (E24 株、特願平 5-211081) である。本菌は、YCW を用いて高活性の酵母溶解酵素を生産できるので酵素生産用培地

が不要である。また培養液をそのまま酵素剤代わりに用いて YCW を可溶化できるので酵素を精製する必要がない。したがって、E24 株を用いることにより安価で効率的な YCW の処理が可能となる。

## 【 0 0 0 4 】

【発明の効果】 E24 株の培養液をそのまま用いて YCW を分解した結果、約 70% の可溶化率が得られた。次に、UASB 装置を用いて、市販酵素 (YL-15、天野製薬株式会社製)、E24 株培養液により可溶化した YCW の嫌気性処理を行った結果、両法により可溶化した YCW の可溶性 COD は、UASB により約 95% 除去され、メタンガスの発生も良好であった。この結果、YCW を E24 株培養液で可溶化することにより安価で効率的な処理が可能であることが確認できた。

## 【 0 0 0 5 】

【実施例】 以下、本発明を実施例に基づき説明する。

## 実施例 1

(E24 株培養液による YCW の可溶化)

方法：静菌処理 (pH を 5.0 に引き下げ、反応温度を 42℃ とし、超音波破碎を行う) を行った培養液により YCW を分解した場合の YCW 分解率、可溶化率の測定方法を図 1 に示した。培養液の超音波処理は超音波ホモジナイザー (Bioruptor、オリンパス光学 (株) 製) を用い、10 分間氷冷しながら行った。分解率及び可溶化率は図 1 中に示した式で計算した。

結果：YCW 分解、可溶化率を算出した結果を表 1 に示した。分解時の温度を 37℃、42℃ にしたもの、pH を 5.0、6.5 としたもの、超音波処理を行ったもののいずれの場合も分解率が 72~76%、可溶化率は 62~70% と高い値となり、培養液をそのまま酵素液として使用できることがわかった。

## 【 0 0 0 6 】 実施例 2

(可溶化した YCW の嫌気性排水処理)

方法：実験に使用した UASB 嫌気性処理装置の概要を図 2 に示した。ビール工場の嫌気汚泥を、5.1 リットルのメタン発酵槽に沈降嫌気汚泥容量が 2 リットルになるように添加し、酸生成槽 (3.1 リットル)、メタン発酵槽を嫌気処理水で満たして運転を開始した。酸生成槽およびメタン発酵槽は、35℃ に保温した。メタン発酵槽出口の処理水は、一部酸生成槽出口に設置した pH 調整槽に循環させ、メタン発酵槽内の上昇流速を 1.0 m/hr となるように循環水量を設定した。酸生成槽、メタン発酵槽の pH は、各々 5.0、6.5 に制御した。原水には、E24 株培養液及び YL-15 による YCW 可溶化液を水道水で希釈し、6100 mgCOD/リットルに調製した液を 4℃ で保存して用いた。COD の測定には、COD<sub>Cr</sub> 測定用試薬 (Hach Company 製 Digestion Solution For COD (high Range)) を用いた。反応には COD REACTOR (Hach Company 製)、測定値の読みとりには DR-100 Colorimeter (Hach Company 製) を用いた。

【0007】結果：YL-15、E24株培養液によるYCW可溶化液を原水とした嫌気性排水処理装置の運転結果を表2にまとめて示した。COD負荷を約10Kg/m<sup>3</sup>/dayに設定し、YL-15およびE24培養液による可溶化YCWによる運転を行った結果、メタンガスの比発生量、可溶性COD除去率ともに良好であった。負荷を約14Kg/m<sup>3</sup>/day(可溶性COD負荷として約10Kg/m<sup>3</sup>/day)に設定した場合も可溶性COD除去率、メタンガス比発生量は良好であり、負荷

を高くしても可溶性CODの処理が可能であることが確認できた。

【0008】以上の結果から、市販酵素あるいはE24株培養液により可溶化されたYCWは、嫌気性排水処理装置により処理可能であることが確認できた。

【0009】

【表1】

試料	Blank	37℃		42℃		37℃ 超音波破碎
		pH6.5	pH5.0	pH6.5	pH5.0	
分解率 %	12.3	75.3	72.3	75.4	74.0	75.3
可溶化率 %	25.4	72.1	63.8	69.5	62.2	64.3

【00010】

【表2】

分解酵素	負荷 KgCOD/m <sup>3</sup> /日	COD除去率 [%]		CH <sub>4</sub> 比発生量 [リットル/g COD]
		可溶性	全体	
YL-15	9.6	93.6	64.6	0.200
YL-15	14.8	95.8	54.2	0.239
E24	11.3	94.4	60.2	0.198
E24	13.9	95.3	66.5	0.246

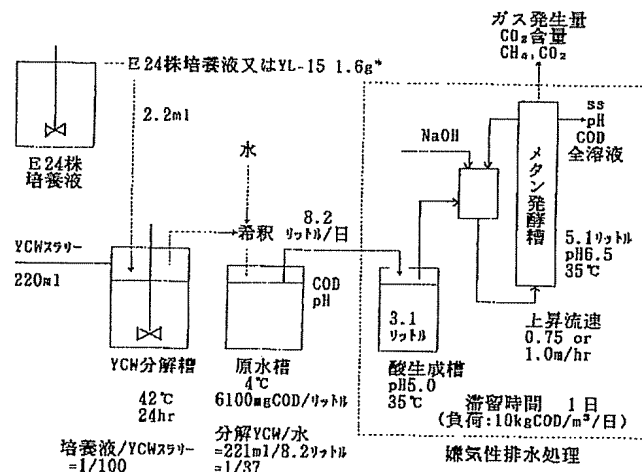
【図面の簡単な説明】

【図2】可溶化YCW処理用嫌気性排水処理装置を示す線図。

【図1】E24株培養液を用いたYCW可溶化率の測定方法を示す線図。

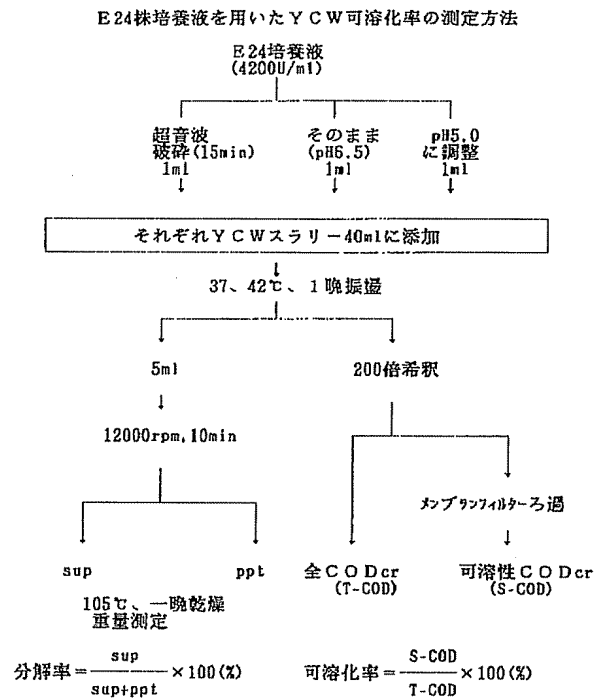
【図2】

可溶化YCW処理用嫌気性排水処理装置



\* E24株培養液の酵素活性=13000U/ml YL-15の酵素活性=28600U/g

【図 1】



フロントページの続き

(51) Int. Cl.<sup>6</sup>  
C 1 2 R    1:01)

識別記号    庁内整理番号

F I

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## PATENT ABSTRACTS OF JAPAN

(11)Publication number : 07-184640

(43)Date of publication of application : 25.07.1995

(51)Int.Cl.

C12N 1/16  
C02F 3/28  
C02F 3/34  
C12N 9/14  
/(C12N 9/14  
C12R 1:01 )

(21)Application number : 05-349045

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(22)Date of filing : 28.12.1993

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## (54) TREATMENT OF RESIDUE OF YEAST EXTRACT

## (57)Abstract:

PURPOSE: To provide a new method for efficiently treating a residue of a yeast extract at low cost by using a cultured solution of a yeast-dissolving enzyme-producing bacterium.

CONSTITUTION: This method for treating a residue of a yeast extract is carried out by solubilizing a residue of a yeast extract using a cultured solution of a yeast-dissolving enzyme-producing bacterium and treating the solubilized solution according to the anaerobic waste water treatment method.

## LEGAL STATUS

[Date of request for examination] 26.09.1995

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number] 2826947

[Date of registration] 18.09.1998

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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CLAIMS

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## [Claim(s)]

[Claim 1] Yeast dissolution enzyme (YLase) Art of the yeast extract residue characterized by solubilizing yeast extract residue (YCW) using the culture medium of a production bacillus, and processing solubilization liquid by anaerobic waste water treatment.

[Claim 2] It is a yeast dissolution enzyme (YLase) as a bacillus according to claim 1. Bacillus OERUSU Coffea from Takao (Oerskovia sp.E24 (the Agency of Industrial Science and Technology biotechnology Industrial Research Institute patent microorganism deposition number, FERM P-13692)) Yeast extract residue art characterized by using it.

[Claim 3] The YCW art characterized by using yeast extract residue as a culture medium which cultivates a bacillus according to claim 1.

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[Translation done.]

## DETAILED DESCRIPTION

## [Detailed Description of the Invention]

[0001]

[Industrial Application] This invention is OERUSU Coffea (Oerskovia). Yeast dissolution enzyme which belongs (YLase) Bacillus from Takao (Oerskovia sp.E24, FERM P-13692) After cultivating using yeast extract residue (YCW) and solubilizing YCW with the culture medium, it is related with processing of YCW characterized by processing by anaerobic waste water treatment.

[0002]

[Description of the Prior Art] As an art of YCW by the conventional technique, it is (1). Ocean dumping or the disposal by reclamation, and (2) Incineration and (3) Direct anaerobic digestion and (4) Although the anaerobic waste water treatment of the solubilization by the yeast dissolution enzyme (YLase) and solubilization liquid can be considered, there are the following troubles. (1) Ocean dumping will be forbidden in the future, and it reclaims land from a viewpoint of environmental protection, and disposal also tends to be restricted. (2) Incineration needs processing of exhaust gas and cannot be satisfied from a viewpoint of environmental protection. (3) Although direct anaerobic digestion is usually used for processing of organic solids, such as sludge, it requires time amount for processing (20 - 30 days). (4) Although it is expected from the conventional technique that it can process with high-speed methane fermentation processors (UASB etc.) by solubilizing with a yeast dissolution enzyme, the costs of an enzyme may become high if a commercial enzyme is used for solubilization of YCW. Moreover, although using a known yeast dissolution enzyme production bacillus is also considered, the culture medium for enzyme production is required. Thus, there are various problems in processing of YCW by the conventional technique, and a cheaper and efficient art needs to be developed.

[0003]

[Means for Solving the Problem] In order to solve this problem, as a result of inquiring wholeheartedly, YCW could be solubilized simple by use of the culture medium of the bacillus from yeast dissolution enzyme Takao, and it found out that this solubilization liquid could be processed by anaerobic waste water treatment. The used bacillus from yeast dissolution enzyme Takao is a bacillus from yeast dissolution enzyme Takao (E24 share, Japanese Patent Application No. 5-211081) which we developed to yeast extract manufacture. Since bacteria can produce the yeast dissolution enzyme of high activity using YCW, its culture medium for enzyme production is unnecessary. Moreover, since culture medium is used instead of an enzyme agent as it is and YCW can be solubilized, it is not necessary to refine an enzyme. Therefore, cheap and efficient processing of YCW is attained by using E24 share.

[0004]

[Effect of the Invention] As a result of decomposing YCW, using E24 share culture medium as it is, about 70% of rate of solubilization was obtained. Next, UASB equipment is used and it is a commercial enzyme. (YL-15, the Amano Pharmaceuticals incorporated company make) As a result of performing anaerobic treatment of YCW solubilized with E 24-share culture medium, the fusibility COD of YCW solubilized by both \*\* was removed by UASB about 95%, and was good. [ of generating of methane ] Consequently, it has checked that cheap and efficient processing was possible by solubilizing YCW with E 24-share culture medium.

[0005]

[Example] Hereafter, this invention is explained based on an example.

Example 1 (solubilization of YCW by E 24-share culture medium)

Approach: The measuring method of YCW cracking severity when the culture medium which performed bacteriostasis processing (pH is reduced to 5.0, reaction temperature is made into 42 degrees C, and ultrasonic crushing is performed) decomposes YCW, and the rate of solubilization was shown in drawing 1 . Sonication of culture medium was performed using the ultrasonic homogenizer (Bioruptor, product made from Olympus Optics), ice-cooling for 10 minutes. Cracking severity and the rate of solubilization were calculated by the formula shown in drawing 1 .

Result: The result of having computed YCW decomposition and the rate of solubilization was shown in Table 1. Although what made temperature at the time of decomposition 37 degrees C and 42 degrees C, the thing which set pH to 5.0 and 6.5, and sonication were performed, it turned out that in any case 72 - 76% and the rate of solubilization serve as a value with as high cracking severity as 62 - 70%, and culture medium can be used as enzyme liquid as it is.

[0006] Example 2 (solubilized anaerobic waste water treatment of YCW)

Approach: The outline of the UASB anaerobic treatment equipment used for the experiment was shown in drawing 2 . The aversion sludge of the Biel brewery was added so that sedimentation aversion sludge capacity might become 2l. at a 5.1l. methane fermentation tub, the acid formation tub (3.1l.) and the methane fermentation tub were filled with aversion treated water, and operation was started. The acid formation tub and the methane fermentation tub kept it warm at 35 degrees C. PH control equipment installed in an acid formation tub outlet was made to circulate through a part of treated water of a methane fermentation tub outlet, and the amount of circulating water was set up so that it might become 1.0 m/hr about the rise rate of flow in a methane fermentation tub. pH of an acid formation tub and a methane fermentation tub was respectively controlled to 5.0 and 6.5. E 24-share culture medium and the YCW solubilization liquid by YL-15 were diluted with tap water in raw water, and the liquid prepared in 6100 mgCOD/the liter was saved and used for it at 4 degrees C. In measurement of COD, it is a reagent for CODcr measurement. (Digestion Solution For COD made from Hach Company) (high Range) It used. DR-100 Colorimeter (product made from Hach Company) was used for the reaction at the readout of COD REACTOR (product made from Hach Company), and measured value.

[0007] Result: The operation result of the anaerobic waste water treatment equipment which used the YCW solubilization liquid by YL-15 and E 24-share culture medium as raw water was collectively shown in Table 2. the result of having set the COD load as about 10 kg/m3/day, and having performed operation by the solubilization YCW by YL-15 and E24 culture medium — the ratio of methane — the yield and the fusibility COD elimination factor were good. Also when a load was set as m3 [ about 14kg./] /day (considering as a fusibility COD load about 10 kg/m3/day), even if the fusibility COD elimination factor and the methane ratio yield are good and it made the load high, it has checked that processing of Fusibility COD was possible.

[0008] From the above result, YCW solubilized with a commercial enzyme or E 24-share culture medium has checked that it could process with an anaerobic waste water treatment equipment.

[0009]

[Table 1]

試料	Blank	3 7℃		4 2℃		3 7℃ 超音波破碎
		pH6.5	pH5.0	pH6.5	pH5.0	
分解率 %	12.3	75.3	72.3	75.4	74.0	75.3
可溶化率 %	25.4	72.1	63.8	69.5	62.2	64.3

[00010]

[Table 2]

分解酵素	負荷 KgCOD/m <sup>3</sup> /日	COD除去率〔%〕		CH <sub>4</sub> 比発生量 〔リットル/g COD〕
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[Translation done.]



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**TECHNICAL FIELD**

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[Industrial Application] This invention is OERUSU Coffea (Oerskovia). Yeast dissolution enzyme which belongs (YLase) Bacillus from Takao (Oerskovia sp.E24, FERM P-13692) After cultivating using yeast extract residue (YCW) and solubilizing YCW with the culture medium, it is related with processing of YCW characterized by processing by anaerobic waste water treatment.

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[Translation done.]

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EFFECT OF THE INVENTION

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[Translation done.]

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TECHNICAL PROBLEM

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[Description of the Prior Art] As an art of YCW by the conventional technique, it is (1). Ocean dumping or the disposal by reclamation, and (2) Incineration and (3) Direct anaerobic digestion and (4) Although the anaerobic waste water treatment of the solubilization by the yeast dissolution enzyme (YLase) and solubilization liquid can be considered, there are the following troubles. (1) Ocean dumping will be forbidden in the future, and it reclaims land from a viewpoint of environmental protection, and disposal also tends to be restricted. (2) Incineration needs processing of exhaust gas and cannot be satisfied from a viewpoint of environmental protection. (3) Although direct anaerobic digestion is usually used for processing of organic solids, such as sludge, it requires time amount for processing (20 - 30 days). (4) Although it is expected from the conventional technique that it can process with high-speed methane fermentation processors (UASB etc.) by solubilizing with a yeast dissolution enzyme, the costs of an enzyme may become high if a commercial enzyme is used for solubilization of YCW. Moreover, although using a known yeast dissolution enzyme production bacillus is also considered, the culture medium for enzyme production is required. Thus, there are various problems in processing of YCW by the conventional technique, and a cheaper and efficient art needs to be developed.

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[Translation done.]

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MEANS

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[Means for Solving the Problem] In order to solve this problem, as a result of inquiring wholeheartedly, YCW could be solubilized simple by use of the culture medium of the bacillus from yeast dissolution enzyme Takao, and it found out that this solubilization liquid could be processed by anaerobic waste water treatment. The used bacillus from yeast dissolution enzyme Takao is a bacillus from yeast dissolution enzyme Takao (E24 share, Japanese Patent Application No. 5-211081) which we developed to yeast extract manufacture. Since bacteria can produce the yeast dissolution enzyme of high activity using YCW, its culture medium for enzyme production is unnecessary. Moreover, since culture medium is used instead of an enzyme agent as it is and YCW can be solubilized, it is not necessary to refine an enzyme. Therefore, cheap and efficient processing of YCW is attained by using E24 share.

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[Translation done.]

## EXAMPLE

[Example] Hereafter, this invention is explained based on an example.

Example 1 (solubilization of YCW by E 24-share culture medium)

Approach: The measuring method of YCW cracking severity when the culture medium which performed bacteriostasis processing (pH is reduced to 5.0, reaction temperature is made into 42 degrees C, and ultrasonic crushing is performed) decomposes YCW, and the rate of solubilization was shown in drawing 1. Sonication of culture medium was performed using the ultrasonic homogenizer (Bioruptor, product made from Olympus Optics), ice-cooling for 10 minutes. Cracking severity and the rate of solubilization were calculated by the formula shown in drawing 1.

Result: The result of having computed YCW decomposition and the rate of solubilization was shown in Table 1. Although what made temperature at the time of decomposition 37 degrees C and 42 degrees C, the thing which set pH to 5.0 and 6.5, and sonication were performed, it turned out that in any case 72 - 76% and the rate of solubilization serve as a value with as high cracking severity as 62 - 70%, and culture medium can be used as enzyme liquid as it is.

[0006] Example 2 (solubilized anaerobic waste water treatment of YCW)

Approach: The outline of the UASB anaerobic treatment equipment used for the experiment was shown in drawing 2. The aversion sludge of the Biel brewery was added so that sedimentation aversion sludge capacity might become 2l. at a 5.1l. methane fermentation tub, the acid formation tub (3.1l.) and the methane fermentation tub were filled with aversion treated water, and operation was started. The acid formation tub and the methane fermentation tub kept it warm at 35 degrees C. PH control equipment installed in an acid formation tub outlet was made to circulate through a part of treated water of a methane fermentation tub outlet, and the amount of circulating water was set up so that it might become 1.0 m/hr about the rise rate of flow in a methane fermentation tub. pH of an acid formation tub and a methane fermentation tub was respectively controlled to 5.0 and 6.5. E 24-share culture medium and the YCW solubilization liquid by YL-15 were diluted with tap water in raw water, and the liquid prepared in 6100 mgCOD/the liter was saved and used for it at 4 degrees C. In measurement of COD, it is a reagent for CODcr measurement. (Digestion Solution For COD made from Hach Company) (high Range) It used. DR-100 Colorimeter (product made from Hach Company) was used for the reaction at the readout of COD REACTOR (product made from Hach Company), and measured value.

[0007] Result: The operation result of the anaerobic waste water treatment equipment which used the YCW solubilization liquid by YL-15 and E 24-share culture medium as raw water was collectively shown in Table 2. the result of having set the COD load as about 10 kg/m3/day, and having performed operation by the solubilization YCW by YL-15 and E24 culture medium — the ratio of methane — the yield and the fusibility COD elimination factor were good. Also when a load was set as m3 [ about 14kg/]/day (considering as a fusibility COD load about 10 kg/m3/day), even if the fusibility COD elimination factor and the methane ratio yield are good and it made the load high, it has checked that processing of Fusibility COD was possible.

[0008] From the above result, YCW solubilized with a commercial enzyme or E 24-share culture medium has checked that it could process with an anaerobic waste water treatment equipment.

[0009]

[Table 1]

試料	Blank	3 7 °C		4 2 °C		3 7 °C 超音波破碎
		pH6.5	pH5.0	pH6.5	pH5.0	
分解率 %	12.3	75.3	72.3	75.4	74.0	75.3
可溶化率 %	25.4	72.1	63.8	69.5	62.2	64.3

[00010]

[Table 2]

分解酵素	負荷 KgCOD/m <sup>3</sup> /日	COD除去率 [%]		CH <sub>4</sub> 比発生量 [リットル/g COD]
		可溶性	全体	
YL-15	9.6	93.6	64.6	0.200
YL-15	14.8	95.8	54.2	0.239
E24	11.3	94.4	60.2	0.198
E24	13.9	95.3	66.5	0.246

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DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]

[Drawing 1] The diagram showing the measuring method of the rate of YCW solubilization using E 24-share culture medium.

[Drawing 2] The diagram showing the anaerobic waste water treatment equipment for solubilization YCW processing.

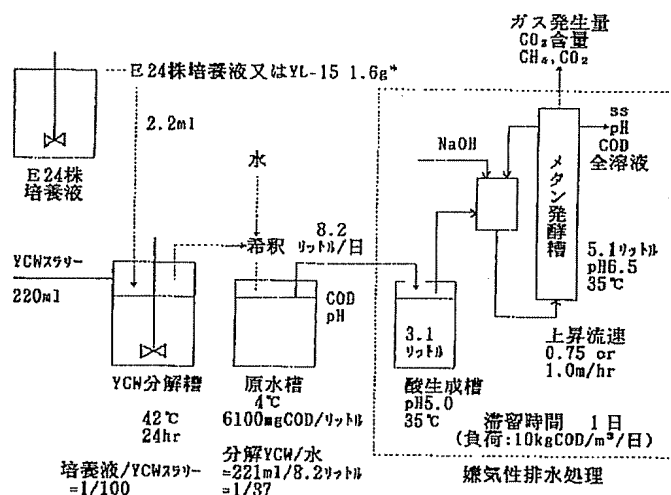
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[Translation done.]

## DRAWINGS

[Drawing 2]

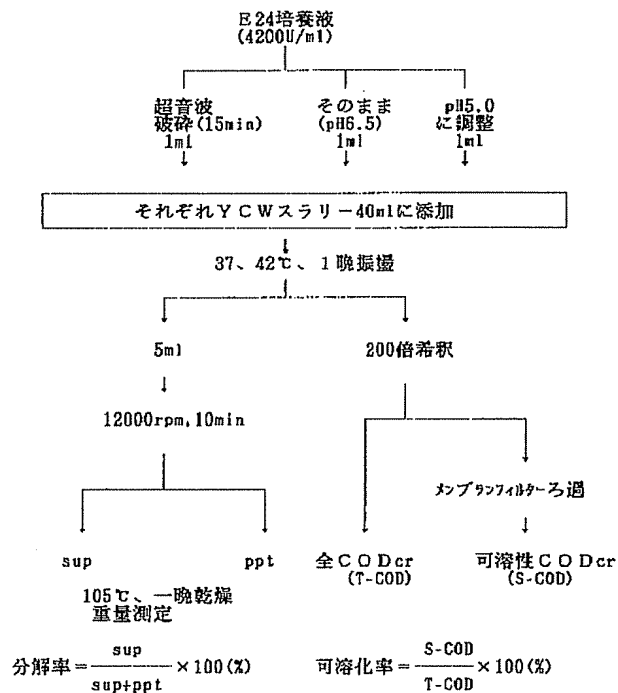
可溶化 Y C W 処理用嫌気性排水処理装置



\* E 24株培養液の酵素活性=13000U/ml    VL-15の酵素活性=28600U/g

[Drawing 1]

E 24株培養液を用いた Y C W 可溶化率の測定方法



[Translation done.]